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German to English Translation
by
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A Procedure for the Analysis of Proteins

Description

The present invention concerns a procedure for the analysis of proteins, wherein, an array of first capture-molecules is employed, which has been specified for peptide-pitope.

Within the state of the technology such procedures are widely known and serve for both the qualitative and quantitative analyses of proteins.

The areas of application for the known procedure are, for example, protein analysis and proof of the presence of protein. An additional, new field of application of the known procedure can be the field of proteomics, this being one branch of research, which dedicates itself totally to the subject of proteins. One aspect of proteomics involves the comparison of the protein-composition in cells, which have been altered by illness, to the normal cells, which have not been so stressed. Such investigations are today, as a rule, carried out by two-dimensional, gel electrophoresis. Another branch of research investigates the spatial structure of the proteins, which, especially for pharmaceutical undertakings, are of interest due to the possibilities of the discovery of newer medicaments.

In the case of the said two-dimensional gel-electrophoresis, a protein mixture, which is to be examined, is placed upon a solid carrier, whereupon, the first separation in regard to the content of the proteins concerning acid and basic amino acidic building blocks is carried out by a pH gradient. An electrical field subsequently separates the proteins in the second direction, so that a spot-sample is created, in which each spot represents one protein. By comparison of the said spot samples, differences in the protein compositions of healthy and ill cells can be investigated. For identification, the protein spots were individually excised and the proteins were caused to degrade into fragments by digestion with specific protease-enzymes. The weights of the said fragments, which remain after this treatment, are characteristic for specific proteins.

In accord with the procedure, mentioned in the introductory passages, for the analysis of proteins, the proof becomes evident, for instance, in array format with protein-specific antibodies. In order to capture such protein specific antibodies, the applicant employed isolated, purified proteins, recombinant proteins or chemically synthesized peptides which are derived from the protein-sequence. The acquiring of the antibodies is done by the immunization of experimental animals with such proteins as act as antigenes, for instance among these would be peptides as well as adjuvants. Otherwise, the possibility exists, of obtaining the antibodies by in-vitro processes as recombined antibodies.

The extraction of the chemically synthesized peptides, from known protein sequences, which, for example are retained in protein data banks, or can be attained from nucleic acid data banks, is accomplished by software programs, about which theoretical predictions regarding the protein structure and a possible antigenicity can be made.

Such programs are described, for example, by Lars Hennig: "WinPep – a Program for the Analysis of Amino Acid Sequences", BIOSPECTRUM 4 (5), 1998, pp. 49-50 or by von Devereaux, et al., "A Comprehensive Set of Sequence Analysis Programs for the VAX", NUCLEIC ACIDS RESEARCH, Vol. 12, 1984, pp. 387-395. With these programs, all possible fragments with a molecular weight, sequence, sequence position and length have been recorded, which have been obtained by a splitting of proteins, principally by proteases or chemical agents. As to a prediction of antigenicity, through structural prognosis, the possibility becomes known, with which an epitope may lie on a protein surface; in this respect refer to Hopp and Woods: "A computer program for predicting protein antigenic determinants", Mol. Immunol., Vo. 20, 1983, pp. 483 - 489. Further literary notice can be found in the World Wide Web under the address: www.expasy.ch.

The antibodies, which have been produced by the use of this technology, recognizes very well the peptide, which has been applied for immunization, but on the other hand, they frequently do not bind on the corresponding peptide-epitope in native protein. The cause for this can be found, for example, in that the peptide-epitope in native protein, because of steric blocking, is not accessible for the antibody, or again this may be because it exists in a post-translated, modified and altered form, which could not be recovered from the data bank. A further reason may be found, in that the peptide-epitope in a native protein is present in a conformation, which prevents antibody binding.

In the case of this technology, it is also disadvantageous, that first, frequently a multiplicity of peptide-specific antibodies are produced from protein epitopes which, subsequently do not, or only poorly, bind on the sought-for protein.

The protein certification with protein-specific antibodies of this kind, can be achieved, in one way, after the separation by SDS-PAGE and transfer to membranes by Westernblot/Immunoblot or, again, by following the ELISA-technology. In the case of the ELISA-technology, the protein to be certified is, without a prior separation from other proteins, can be bound directly from a complete protein solution onto a highly-specific antibody, which is immobilized on a solid carrier. The protein solution is thereafter, rinsed from the solid carrier and the bound, residual protein which remains on the carrier can be proved.

This certification is done with the aid of either marked double antibodies, which are specifically for the bound protein or with the help of competition of the analyte proteins with marked analyte proteins added to the solution in specified doses. What is being put to use, in this indirect proving method, is that non-marked analyte protein, from the sample and added marked analyte protein, in a defined manner, regulated by the law of mass action, compete around the available binding sites. From this, it becomes evident, that the bound, marked analyte protein, is to be found in inverse proportion to the quantity of the non-marked protein in the sample.

This procedure possesses disadvantages. First, the already mentioned fault, that namely, the production of the specific antibody leads to a multiplicity of antibodies, which do not, or only poorly bind onto the protein to be investigated. Second, the application of the Westernblot-technology has the additional disadvantage, that the protein mixture, which has been isolated by the SDS-PAGE procedure, can be tested with differently tagged antibodies, in order to verify a degree of difference of the bound antibodies attached to different proteins.

Even in the case of the ELIZA-technology, the restriction is, that only one antibody per analysis-cavity can be immobilized and subsequently incubated with the protein solution. This technology requires considerable quantities of sample, since every analysis-cavity must be incubated with an aliquot of the protein mixture. In the case of the known method, another disadvantage is, that the proof of the bound proteins require specific double antibodies for each protein to be certified, or else require large quantities of marked analyte proteins for competition experiments.

As has already been stated, the applicant employed protein-specific antibodies with different specificities also functionally immobilized in the array format in rows and columns on a carrier material. The sought-for proteins were marked and subsequently incubated with the antibodies on the array. The proteins in the solution, which supply the antigene for the immobilized antibodies, bind, under these circumstances, onto the antibodies which were specified for them, so that a positional-specific protein binding was created.

Because of the known binding specifications of the immobilized antibodies and the known positions of the individual antibodies in the array, the bound amount of the individual proteins can be determined in parallel. For this purpose, by a position-determining detection, the bound protein amount can be fixed with the aid of the markings on the proteins.

Besides the qualitative, parallel certification of various proteins present in the sample solution, it is possible, by the addition of standard proteins to also attain a quantitative determination of the analyte proteins.

Counter to the procedures described above, at this point, it is of advantage, that in the same sample and in one cavity, several proteins can be detected in parallel. However, the disadvantage here resides in the fact that the proteins to be analyzed must be marked, for which purpose corresponding markings on specific functional groups of individual amino acids of the proteins must be introduced. The efficiency of such marking reactions is very different for different proteins. The efficiency is affected by the immediate, individual micro-neighborhood of a functional group, as well as by steric-blocking and pH variation in the immediate proximity of the functional group by neighboring groups, salts, solvents and the like. Experience has shown that this can be interpreted in that the reactivity of chemically identical functional groups can be very different in a single protein, so that a quantitative conversion of defined functional groups becomes very difficult.

Moreover, the marking reaction can lead to a situation, wherein modification occurs with amino acids within a recognized epitope from a specific antibody, which leads to a loss of binding between a protein epitope and an antibody. On this account, however, the protein which has been so marked, is no longer capable of being identified by the described test procedure.

Thus, with this background, the purpose of the present invention is to develop the procedure mentioned in the introductory passages in such a manner, that a quantitative and/or a qualitative analysis of proteins becomes possible in a simple, rapid and reliable manner.

In accord with the originally mentioned procedure, this purpose is achieved by the three steps:

a) degradation of the proteins to be analyzed or of a mixture of the proteins to be analyzed to the peptide-fragments corresponding to the peptide-epitopes,

b) incubation of the arrays with the peptide fragments, and

c) certification of the peptide fragments bound on the first capture-molecules.

The purpose of the invention is completely achieved in this manner. The invention is based on the surprising recognition of the inventor, that the capture-molecules immobilized in the array are not in accord with the state of the technology as to proteins, but rather, for example, are incubated, after an enzymatic degradation of the proteins to individual peptide fragments, which correspond to the peptide-epitopes, which have been added for the generation of the specific first capture-molecules.

In the case of this invented procedure, first, it is to advantage, that the secondary and the tertiary structure of the proteins to be analyzed play no role. The first capture-molecules recognize the corresponding peptide fragments with great assurance, since they were generated from corresponding peptide-epitopes. These epitopes can be directly preselected from data banks. An additional and complex preselection of secondary and tertiary structure is not required. In this way, the new procedure is not only reliable, but also simple and rapid to execute, since the first capture-molecules reliably recognize the proteins by the proven peptide fragments.

An addition advantage, can be found in the fact, that each capture-molecule selects its optimal antigene, because the synthetic peptide, which was used for the making of the capture-molecules, is identical to the peptide fragment to be proven. In this way, the disadvantages present in the state of the technology are avoided, in that in the case of proteins, it cannot be predicted as to whether or not a peptide specific antibody can also select its own native protein. In other words, this means, that the losses upon the first capture-molecules is clearly less than those of the state of the technology.

Since the arrangement of the different first capture-molecules in the array is known, it becomes possible, that by a position-sensitive detection method, which can be automatized, a qualitative identification and a quantitative determination of the fragments, and hence of the proteins can be accomplished. The found peptide pattern correlates namely directly with the protein pattern, which gave itself a determination upon the making of the arrays, since these were analyte proteins. Beyond this, it is

also possible, that the found peptide pattern can be screened with simple, rapid algorithms from a DNA-data bank.

In this way, a rapid and a reliable statement becomes possible, as to which proteins are present in a sample solution, which, for example, can be included in diagnostics. Further with the new procedure it becomes possible, for the first time, to make a mutation analysis on the level of proteins.

An additional advantage of the invented procedure lies in its speed, for the smaller molecular-count fragments permit a more rapid run of binding assays than is the case with high count molecular proteins. Thus there arises a kinetic acceleration of the assays, whereby a similar kinetic of the analyte binding leads to a simpler establishment of the optimal conditions for the assays, than is this the case for the greatly varying kinetics, which exists for the analyte proteins in their greatly variable molecular weights.

Another advantage of the new procedure lies therein in that the degradation of the proteins to be analyzed to obtain the peptide fragments can be done in a complete and defined manner, and that it is the fragments and not the proteins, which permit themselves to be quantitatively marked at particular functional groups. This leads, in turn, to a situation, wherein the proof of the peptide fragments is made not only qualitatively, but also quantitatively, without the necessity of turning to the secondary and the tertiary structures. Advantages can now be found in that:

- if, subsequent to the incubation, non-bound fragments are washed away,
- if, in a known manner, the specificity of the procedure is increased, and
- in that the peptides are marked, after the incubation.

Since specific, complete marking is possible of the peptide fragments as defined, functional groups, consequently, a quantitative determination of each bound peptide-epitope is permissible. This is accompanied by the advantage, that a complete marking of the peptide fragments is possible in an easier and more reproducible way, than is the case of a marking of proteins, as this is carried out in the state of the technology.

Further, it is advantageous, if the confirmation by marked second capture-molecules is done, wherein the said capture-molecules recognize the peptide fragments which are specifically bound to the first capture-molecules.

In this case, it is of advantage, if the peptide fragments need not be marked, so that the procedure, as a whole, can be carried out more rapidly, for marked second capture-molecules are able, in a single procedure-step, to be assembled for availability in a greater quantity, whereby, on this account, a plurality of invented analysis procedures with various samples of proteins can be carried out, which samples, respectively, can be degraded to form the corresponding peptide fragments.

A further advantage of this measure can be found therein, in that the selectivity is increased, since bound, unspecified peptide fragments from the first capture-molecule are not recognized, since the second capture-molecule cannot bind onto these complexes.

Further, it is advantageous, if the second capture-molecules are initiated from complexes derived from the first capture-molecules, whereupon, subsequently peptide-epitopes are produced thereon.

In this matter, it is further of advantage, when very specific second capture-molecules are produced, which, additionally, are made with a higher yield quota.

In total, preference is shown, if the first capture-molecules are produced from peptide-epitopes, which were purposely made in consideration of peptide fragments , which emerged from the degradation of the proteins to be analyzed.

These measures are connected with an entire series of advantages. To begin with, the first capture-molecules produce themselves as compared with the linear peptide-epitope with a higher yield quota, since the peptide-epitopes for the production of, or the isolation of the capture-molecules are completely identical to the peptide-epitopes to be analyzed. This leads to the situation, that in the production of the first capture-molecule, considerably less reject material is accumulated, than is the case in the production of protein-specific capture-molecules. Such peptide-epitopes for the production of, i.e. the isolation of the capture-molecules, can be made quickly and economically and fully analytically characterized, which forms a substantial advantage over the state of the technology.

By this measure, additionally, the specificity of the new procedure is increased. Epitopes occurring in various proteins can, respectively, be augmented by additional protein specific epitopes in such a manner, that the combination of those epitopes confirmed on the array can clearly defined without doubt, as analyte proteins. In this way, not only the specificity and redundance of the new procedure is increased, but also a desired optimization of the proof of specific proteins becomes possible.

The peptide-epitopes can, for this purpose, by chemical synthesis or enzymatic degradation, be produced from known proteins, so that these can be fully analytically characterized by low-cost standard methods.

It is additionally preferred, that the desired, produced peptide-epitopes be selected from potential peptide-epitopes of the protein to be analyzed .

It is advantageous, that only peptide-epitopes are made, from which even capture-molecules can be produced. This measure leads also to time saving and synthesis economies in the production of the first and/or the second capture-molecules. As to "potential", what is to be understood, in this case, are all possible epitopes of a protein, including such as are not to be found on the surface of the protein and up to now have not been used for the generation of capture-molecules. Thus it is again of value, in that per protein, many more possible epitopes can be given consideration, than is the case in the state of the technology, so that, for example, in a case of very similar or nearly related proteins, and much greater selection of peptide-epitopes stand as available for possible differentiation between the proteins.

Moreover, it is favorable, that only such peptide-epitopes be made, which are also specific for one protein, or else, are specific for very few proteins.

It is of advantage, if the potential peptide-epitope is derived from known amino acid and/or nucleic acid sequences of the proteins to be analyzed.

It is again of advantage, that the prediction of the peptide-epitope be done from certifiable proteins of DNA or protein data banks, without consideration of the protein-tertiary-structure. A simple prediction of the protein separative locations for the agents added to initiate the specific protein splitting suffices, wherein the selection of one or more peptide-epitopes is done with the greatest possible specificity for the analyte protein. In an optimal case, the epitope occurs only in the analyte protein.

Further preference is given, when, as potential peptide-epitope such sequence sections are chosen, which remain upon the degradation of the proteins to be analyzed.

In the case of this measure, time-saving is of value, so that namely only such capture-molecules should be produced, for which also peptide fragments can be found, whereby the potential peptide-epitopes must be guardedly observed, in regard as to whether they are specific for one or for a few analyte proteins and that the possibility of their production is highly likely.

The prediction of difficultly accessible peptide sequences by chemical synthesis can be brought in as an additional criterion for the choice of the peptide-epitope. The classical prediction of immunogenic, linear peptide sequences, by which, because of the hydropathy profile (Kyle and Doolittle, "A Simple Method for Displaying the Hydropathic Character of Protein", J. MOL., BIOL 157 (1982), pp 105-132) and the prediction of β-loop Inducing Sequence Sections, (Chou and Fasman, "Prediction of Protein Conformation", BIOCHEMISTRY 13, 1974, pp 222-245) potential linear peptide-epitopes in protein structures were identified. This is not required here, since following denaturing and degradation of the analyte protein all peptide fragments, independently of their position in the protein structure present potential peptide-epitopes.

This leads to a larger choice of potential peptide-epitopes, and therewith to a greater possibility to find epitopes specific to proteins to be analyzed (i.e. analyte proteins), than by the arrays of capture-molecules, which have been incubated with complete analyte proteins.

Further, preference is given, if the desired, produced peptide-epitopes are so marked, and/or modified, as are the peptide fragments after, or during the degradation of the protein to be analyzed.

In the case of this measure, it is of value, in that it leads to a high specificity of the first capture-molecules, since already, with the production of the peptide-epitope used for the production of the first capture-molecule, consideration was given as to whether or not, the peptide fragments after/during the degradation of the protein to be analyzed were marked or modified.

An additional advantage is to be found in the desired inset of markings by automatic chemical synthesis, whereby a rapid, economical, and completely defined marking is possible, which is simpler, less costly and more capable of reproduction that is the marking of the entire protein.

When this is done, it is preferred, that the intended, produced peptide-epitopes are provided with posttranslational modifications.

In the case of this measure, it is of advantage, that the proteins to be analyzed can be screened onto posttranslational modifications. In that way, it is possible that, for example, peptide-epitopes from the source of potential phosphor-ylized positions of a protein are made as synthetic phosphor-peptides and as nonphosphor-ylized peptides, in order to generate capture-molecules instead of the respective peptide-epitope. An array from capture molecules of this type, which recognize the different phosphor-ylated and/or non-phosphor-ylated peptide-epitopes, can then be added, in order that the phosphor-ylation condition of one or more proteins is quantified directly from a protein mixture. Other modifications, which can be screened in this way, include modified amino acids, glycopeptides and the like.

Further, in an advantageous way, the desired produced peptide-epitopes, in comparison to the potential peptide-epitopes, at least possess one amino acid exchange and/or at least one deletion of an amino acid.

In the case of this measure, it is of advantage, that a single nucleotide polymorphism can be recognized, since the exchange, that is to say, the deletion of individual amino acids in proteins, can be detected and quantified. For this purpose, epitopes, from the area of the exchange of amino acids, are made in order to generate specific capture-molecules using these epitopes. After the enzymatic degradation of the corresponding proteins and, if necessary, the marking of the generated peptide fragments as well as incubation with the array, it becomes possible that the various peptides made possible by the single nucleotide-polymorphism along with the different amino acid exchanges and deletions can be certified and quantified directly on the said array.

In general, it is preferred, if the capture molecules or each capture-molecule, are capture-molecules which can be produced by chemical synthesis or mutation from recombinant binding-domains. A capture module can be an antibody, an antibody fragment, a peptide-aptamer, or the like.

Additionally, it is advantageous, if the binding specificity of the capture-molecule is determined by binding tests with peptides in individual amino acids or with peptides which vary in their length. Thereby quickly and economically is enabled a complete characterization of the specificity of the generated capture-molecule for the avoidance of cross-reaction activity against other epitopes. In the case of proteins themselves, this cannot be accomplished within a reasonable cost in time and money.

This can, in accord with the invention be done either in the array with synthetic peptides, respectively in a defined sequence or it may be carried out with peptide data-banks for individual, immobilized capture-molecules and subsequent identification of bound peptides.

With this background, the present invention concerns further, a capture-molecule, especially a capture-molecule for use in a procedure of the present kind, which is bound particularly on peptide-epitopes which represent the peptide fragments, in which the proteins to be analyzed can be deraded and

whereby, advantageously, the peptide-epitope within the protein itself, does not have access to the capture-molecule.

In the case of this capture-molecule, which is not to be found in the state of the technology, it is of advantage, that the said capture-molecule can, in a very specific manner, differentiate between very similar or nearly related proteins, since the number of the peptide-epitopes, which do not lie on the surface, and which peptide-epitopes are also, for example, masked in the protein itself, is very large. In this way, a great number of peptide-epitopes stand available, which, in the state of the technology could not previously be made use of, in order to generate specific antibodies, which could be used at the protein level for diagnostics, mutation analysis and the like.

Additionally, the present invention concerns a capture-molecule, that specifically binds on a complex of a said capture-molecule and the corresponding peptide-epitope.

This kind of second capture-molecules can be used in order to increase the specificity of the confirmation tests, as this has already been thoroughly explained in connection with the individual procedure steps.

It is obvious, that the features and advantages presently explained, and those following, need not be in the given combination, but can also be self sustaining, or be usable in other combinations, without leaving the framework of the present invention.

The basic principle of the procedure for the quantitative and/or qualitative analysis of proteins is to be found therein, in that a array of first capture molecules specifically for peptide-epitopes is applied, whereby not proteins, but, as example, peptide fragments which are residual after the enzymatic splitting of the proteins, which represent the peptide-epitopes, with which in array format, immobilized protein-specific capture-modules are confirmed.

Further in this matter, next, potential peptide-epitopes or protein-sequences were theoretically predicted in protein or gene-sequence data banks. Since, by the enzymatic or chemical splitting of the proteins to be confirmed, individual epitopes could be in a disseminated state, it is a requirement for the prediction of potential peptide-epitopes, that the interface specificity of the protease used for the protein degradation must be taken into consideration. Potential peptide-epitopes, which were theoretically predicted , according to these criteria for the individual proteins, were produced by chemical peptide synthesis and marked on the specific functional groups, for example, the amino-function of the N-terminal or the ε-amino group of Lysin, for example with fluorophor, biotin and the like.

These peptide-epitopes were then used for the well known production of antibodies by immunization. The capture-molecules, made in this way, which, in the simplest case, could also be antibodies, were then immobilized in rows and columns on the array.

By enzymatic or chemical degradation reaction with specific proteases, such as trypsin, endoprotease lys C, etc., all proteins of the entire protein mixture to be analyzed are recovered as the

protein fragments representing the peptide-epitopes, which are then incubated with the array, and are certified with the aid of their binding onto the first capture-molecules.

For confirmation, it is possible, that first, the obtained peptide fragments can be provided with a marker, such as fluorophore, biotin, and the like, marking onto the same functional groups as were the synthetic peptide-epitopes, originally added for the production of the specific capture molecule. The corresponding, marked peptides are incubated on the antibody arrays, the non-bound peptide fragments are washed away and the bound peptide fragments are certified by their markings in a positionally specific manner. Since a complete marking of the peptide is possible, it is possible that a quantitative determination of each bound peptide-epitope can be carried out.

Otherwise, even non-marked peptide-epitopes can be proven, if a marked second capture-molecule is added, which, in the simplest case can be a double antibody, which recognizes the peptide which is bound onto the immobilized antibody. This double antibody is generated by the bound peptide, which is on the immobilized antibody, whereby a drastic increase of the selectivity occurs, since only the specific peptide fragment is recognized on the immobilized first antibody and in that place, unspecifiedly bound peptide fragments from the second antibody are not bound.

In this way, only peptides, which present potential immunogens and are not fragmented during the degradation of the protein, are employed for the immunization, which leads to a savings in the chemical peptide synthesis and the production of specific capture-molecules, since highly affine, peptide-specific capture-molecules with a high result quota can be produced. This leads to a reduced rejection of capture-molecules than is the case with the production of protein specific capture molecules.

The binding specificity of the obtained capture molecules, in this case, also antibodies in an array format, can be determined by a binding test with variably defined, synthetic peptides in individual amino acids. Alternatively, it is also possible, that synthetic peptide supply banks, which contain all theoretically possible peptide sequences, including individual, that is to say, antibodies incubated on affinity chromatographic columns, Those bound peptides, derived out of the peptide mixture, after denaturing of the antibody, can be eluted and identified by mass-spectroscopy or by the Edman-degradation.

With the new procedure, it is possible that proteins from the degradation of cells, from bodily fluids, or tissues can be examined, not only quantitatively, but, for example, also on post-translational modifications as well as single nucleotide polymorphism.

CLAIMS

Claimed is:

1. A procedure for the analysis of proteins, wherein an array of first capture molecules specific for peptide-epitopes is added, characterized by the steps:
   - the degradation of the proteins to be analyzed or the degradation of a protein mixture containing the proteins to be analyzed reduced to peptide fragments representing corresponding peptide-epitopes,
   - incubation of the array with the peptide fragments, and
   - certification of the peptide fragments on the first capture molecule.

2. A procedure in accord with claim 1, therein characterized, in that, following the incubation, unbound peptide fragments are removed by washing.

3. A procedure in accord with claim 1 or 2, therein characterized, in that the peptide fragments are marked before the incubation.

4. A procedure in accord with one of the claims 1 to 3, therein characterized in that the confirmation regarding marked second capture-molecules is attained, when said second capture molecules specifically recognize peptide fragments bound on the first capture molecule.

5. A procedure in accord with claim 4, therein characterized, in that the second capture molecule is produced as evolving from complexes of the first capture molecule and the thereon bound peptide-epitopes.

6. A procedure in accord with one of the claims 1 to 5, therein characterized, in that the first capture molecules are produced from the peptide-epitopes, which were made with consideration given to the peptide fragments, which were created upon the degradation of the proteins to be analyzed.

7. A procedure in accord with claim 6, therein characterized, in that the desired, produced peptide-epitopes were selected from potential peptide-epitopes of the protein to be analyzed.

8. A procedure in accord with claim 7, therein characterized, in that the potential peptide-epitopes were developed from known amino-acids and/or nucleic acid sequences of the protein to be analyzed.

9. A procedure in accord with claim 7 or 8, therein characterized, in that, as a potential peptide-epitope, such sequence sections were selected, which remained, after the degradation of the protein to be analyzed.

10. A procedure in accord with one of the claims 6 to 9, therein characterized, in that the desired, produced peptide-epitope is so marked and/or so modified, as is the peptide fragments after/during the degradation of the proteins to be analyzed.

11. A procedure in accord with one of the claims 6 to 10, therein characterized, in that the desired, produced peptide-epitope is provided with post-translational modifications,

12. A procedure in accord with one of the claims 6 to 11, therein characterized, in that the desired, produced peptide-epitope possesses, with the potential peptide-epitope at least one amino-acid exchange and/or a deletion of an amino-acid.

13. A procedure in accord with one of the claims 1 to 12, therein characterized, in that the capture-molecules, or each capture-molecule, an antibody, an antibody fragment, a peptide-aptamer, or the like is a capture-molecule which can be produced by chemical synthesis or mutation from recombinant binding-domains.

14. A procedure in accord with one of the claims 1 to 13, therein characterized, in that the binding specificity of the capture molecules is determined by binding tests with peptides in individual amino acids or with peptides of varying length.

15. A capture molecule, especially for the application in a procedure in accord with one of the claims 1 to 14, therein characterized, in that it specifically binds onto peptide-epitopes, which represent peptide fragments, in which the proteins to be analyzed can be degraded.

16. A capture molecule in accord with claim 15, therein characterized, in that the peptide-epitope in the protein itself, is not accessible by the capture molecule.

17. A capture molecule, which specifically binds on a complex from a capture molecule in accord with claim 15 or 16 and the corresponding peptide-epitope.

Summary

In the case of a procedure for the analysis of proteins, an array of first capture molecules, specifically for peptide-epitopes is added. The proteins to be analyzed or a protein mixture which contains one of the proteins to be analyzed, is degraded to peptide fragments which represent the peptide-epitopes, whereby the array of capture molecules with the peptide fragments is incubated. Subsequently, the peptide fragments bound onto the capture molecules are certified.